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(54) Title: CONTROLLED RELEASE OF PHARMACEUTICALLY ACTIVE SUBSTANCES FROM COACERVATE MICROCAPSULES

## (57) Abstract

A controlled-release pharmaceutical composition comprising a pharmaceutically active substance, encapsulated in the form of microspheres by a coating of a crosslinked coacervate of gelatin and chondroitin sulfate, as well as processes for the preparation thereof.

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**CONTROLLED RELEASE OF PHARMACEUTICALLY ACTIVE  
SUBSTANCES FROM COACERVATE MICROCAPSULES**

**BACKGROUND OF THE INVENTION**

*1. Field of the Invention*

5        This invention relates to coacervate-based microcapsules which include pharmaceutically active substances as well as to a method of providing controlled release of such substances *in vivo*. The microcapsules of the invention are useful to prepare  
10      and deliver drugs, biologicals, enzymes, and pharmaceutically active substances.

*2. Description of Related Art*

      A controlled delivery system which will dependably release a biologically active substance (e.g., a  
15      pharmaceutical agent) *in vivo* into a biological fluid (e.g., fluids of gastrointestinal tract) or into body tissues has remained an elusive goal. Biologically active endogenous substances such as cytokines, hormones, enzymes, and antibodies are usually less  
20      stable than conventional synthetic drugs in the physiological environment. The controlled delivery system thus provides a suitable means to administer these endogenous substances to a host.

      A common approach to providing controlled release  
25      of the active substance is the encapsulation of the active substance within a polymer matrix (e.g., that made of hydrophilic polymers). While some combinations of a drug and particular polymers provide a suitable drug release profile, they require a relatively large  
30      quantities of synthetic materials to be delivered to the body. This is not desirable since such materials or their metabolites *in vivo* may not be biocompatible or even may have toxicity such as cytotoxicity or mutagenicity. Furthermore, encapsulation involves the  
35      use of organic solvents and/or heat, both of which can be harmful to peptide or proteinous drugs, not to mention the above-described endogenous substances.

Complex coacervation is a process of separation of colloidal solutions into two or more immiscible liquid phases. When oppositely charged polyelectrolytes are brought to contact in aqueous medium, a spontaneous  
5 phase separation occurs with the result of formation of coacervates. The coacervate is a phase where colloids (e.g., polymers) are concentrated.

Coacervation has been employed to encapsulate water insoluble biologically active substances. For example,  
10 U.S. Patent No. 4,794,000 discloses a method for preparing a pharmaceutical composition for oral administration which is based on two phase coacervate system where erythromycin is included as the active ingredient.

15 U.S. Patent No. 5,051,304 discloses microcapsules formed by coacervation between gelatin and a chemically depolymerized polysaccharide wherein a variety of water immiscible substances can be included.

While these and other references generally teaches  
20 the applicability of the coacervation technique to a delivery system in the form of a coacervate, the coacervate formation to encapsulate a particular substance of interest can only be achieved by carefully controlling the phase separation conditions such as the  
25 choice and concentrations of suitable polyelectrolytes, pH and temperature.

Rheumatoid arthritis, which affects 3-4% of the population, is characterized by inflammation and pain of joints. Although the etiology of rheumatoid  
30 arthritis is not well elucidated, both steroid and non-steroidal therapy have been to alleviate the symptoms of this illness. However, gastrointestinal irritation, including ulcers, is a side effect commonly associated, to one degree or another, with antiinflammatory agents.  
35 In order to avoid such side effects and to enhance drug concentrations in synovial sites, intra-articular injection is considered a viable method of

administration of an anti-inflammatory agent. There appears to be no literature available which suggests coacervate-based microcapsules as a intraarticular form of administration of the anti-inflammatory agent.

5       The present invention permits desirable chemotherapy, including anti-inflammatory therapy, by providing controlled release of a pharmaceutically active substance into the body while preventing or ameliorating undesirable side effects associated with  
10 administration of the substance by a known delivery system.

#### **SUMMARY OF THE INVENTION**

In one aspect thereof, the present invention provides a pharmaceutical composition for the  
15 controlled release of a pharmaceutically active substance comprising a therapeutically effective amount of the pharmaceutically active substance in the form of microspheres, encapsulated by a coating of a crosslinked coacervate of gelatin and chondroitin  
20 sulfate.

In another aspect, the present invention also provides a process for preparing the above-described pharmaceutical composition which comprises the steps of:

- 25       (a) providing a gelatin aqueous solution;  
         (b) providing a chondroitin sulfate aqueous solution;  
         (c) adding a therapeutically effective amount of a pharmaceutically active substance either to the  
30       solution in step (a) or to the solution in step (b);  
         (d) mixing the gelatin and chondroitin sulfate solutions to form a coacervate suspension;  
         (e) adding a crosslinking agent to the coacervate  
35       suspension to crosslink the coacervates, the coacervates encapsulating the pharmaceutically active substance; and

(f) incubating the coacervate suspension to form microspheres and recovering the microspheres.

In further aspect, the present invention provides a method of releasing a pharmaceutically active substance *in vivo* in a controlled manner which comprises administering the above-identified pharmaceutical composition to a host and degrading the coacervates by the action of a gelatin-digesting enzyme.

In still further aspect, the present invention provides an encapsulated pharmaceutically active substance. The above features and advantages of the present invention will be more fully understood by reference to the following detailed description and the drawings.

#### **BRIEF DESCRIPTION OF THE DRAWINGS**

FIGURE 1 is a photomicrograph of the microspheres prepared in the practice of the present invention.

FIGURE 2 shows the *in vitro* release kinetics of SCR1 when incorporated in the pharmaceutical composition according to the present invention.

FIGURE 3 shows the activity of released SCR1 in medium with time in the practice of the present invention.

#### **DETAILED DESCRIPTION OF THE INVENTION**

In this disclosure, the term "microcapsules" refers to microspheres wherein a pharmaceutically active substance is encapsulated by a coating of coacervates. Specifically useful in the present invention is a solid microsphere which is a matrix type rather than a core-shell type. If the pharmaceutically active substance is a charged molecule such as protein, the molecule is likely to participate in the complex coacervation process to form the microsphere wherein the substance is entangled with the microsphere matrix.

As applied in the present invention, the term "pharmaceutically active substance" is intended to encompass any substance that will produce a

therapeutically beneficial pharmacological response when administered to a host, both human and animal. Suitable substances include, but are not restricted to analgesics, antibacterials, antifungals, immunosuppressants, anti-inflammatories, and anti-cancer agents. Some non-limiting examples of antibacterials include penicillins, cephalosporins, tetracyclines, quinolones, and aminoglycosides. Representatives of anti-inflammatories include hydrocortisone, colchicine, ibuprofene, indomethacin, and piroxicam. More than one pharmaceutically active substance may be included, if desired, in the pharmaceutical compositions of the present invention. The active substances may be water-soluble or water-insoluble and may include those having a high molecular weight proteins, peptides, carbohydrates, glycoproteins, lipids, and glycolipids. The process of the present invention is particularly suitable for encapsulation of these high molecular weight, susceptible biomacromolecules.

The pharmaceutically active substance can be employed in the present invention in various forms, such as molecular complexes or pharmaceutically acceptable salts. Representative examples of such salts are succinate, hydrochloride, hydrobromide, sulfate, phosphate, nitrate, borate, acetate, maleate, tartrate, salicylate, metal salts (e.g., alkali or alkaline earth), ammonium or amine salts (e.g., quaternary ammonium) and the like. Furthermore, derivatives of the active substances such as esters, amides, and ethers which have desirable retention and release characteristics but which are readily hydrolyzed *in vivo* by physiological pH or enzymes can also be employed.

As used herein, the term "therapeutically effective amount" means that the amount of the pharmaceutically active substance is of sufficient quantity to induce

desired pharmacological effect. The amount can vary greatly according to the effectiveness of a particular active substance, the age, weight, and response of the individual host as well as the nature and severity of the host's symptoms. Accordingly, there is no upper or lower critical limitation upon the amount of the active substance. The required quantity to be employed in the present invention can readily be determined by those skilled in the art.

As used herein with respect to the pharmaceutically active substance, the term "controlled release" is intended to mean that the present composition require at least an hour to release a major portion of the active substance into the surrounding medium, e.g. about 1-24 hours or even much longer.

In accordance with the present invention, the pharmaceutically active substance is encapsulated in a coacervate which is reinforced by crosslinking. Components that may be used to form the coacervate comprise anionic and cationic molecules. By "cationic" or "anionic" molecule is meant a molecular which, under the conditions of coacervation, carries a net positive or negative charge. Many such molecules are amphoteric and contain both acidic and basic groups. Under prevailing pH conditions, these molecules can be either cationic or anionic. Cationic molecules include albumin, collagen, elastin, gelatin, and globulins. Anionic molecules include chondroitin sulfate, dermatan sulfate, heparin, heparan sulfate, hyaluronic acid, keratan sulfate, polygalacturonic acid, and polyglucuronic acid. The choice of the cationic and anionic molecules depends on the final or intended use of the present compositions and they are expected to possess a high degree of biocompatibility. Preferably, gelatin is used as the cationic molecule in the present invention. Collagen is also preferred as the cationic molecule. Chondroitin sulfate is preferably used as



the anionic molecule. Chondroitin sulfate is a mucopolysaccharide found in skeletal and soft connective tissues. The crosslinking is preferably effected by use of a gelatin-hardening agent such as glutaraldehyde. Epichlorohydrin and acrolein can also be used as the crosslinking agent.

Typically, in preparing the coacervates of the present invention, both types of components are brought into contact in an aqueous medium under predetermined mixing conditions. The mixing can conveniently be carried out using equivolumes of an aqueous gelatin solution and an aqueous chondroitin sulfate solution. Preferably, gelatin is present in the aqueous solution in a concentration of from about 1% to about 10% (w/vol). Chondroitin sulfate is present in the aqueous solution in a concentration of from about 0.05% to about 2% (w/vol). Outside these concentration ranges, the formation of coacervates appears to be adversely effected.

The temperature to be employed in forming coacervates may vary somewhat with the particular method employed. Thus, a temperature from ambient temperature to about 50°C can normally be used. However, the size of coacervates is susceptible to the temperatures of the two components when they are mixed. The higher the temperature of the gelatin solution, the smaller become the coacervates formed. The pH to be employed in forming coacervates can also vary over a fairly wide range. The yield and size of coacervates are influenced by the pH. Preferably, the pH of from about 3.5 to about 6.5 (the latter of which corresponds to the isoelectric point of gelatin) is used. The pH of the gelation solution predominates over that of the chondroitin solution in determining the pH of the mixture of the two. Optimum results in terms of both the size and yield of the coacervates are obtained at a pH of about 5.0.

The desired amount of the pharmaceutically active substance is added either to the gelatin solution or to the chondroitin sulfate solution prior to the formation of coacervates. If the active substance is water-soluble, a solution results. On the other hand, if the active substance is water-insoluble, an emulsion or suspension results.

Upon mixing of the gelatin solution and the chondroitin sulfate solution with stirring, coacervation takes place in the reaction medium to form coacervates of microsphere. Since a coating of coacervate (walls) is generally too fragile for coacervates to be isolated, the crosslinking of the coacervates is necessary. The crosslinking is preferably carried out by adding to a suspension containing stabilized coacervate-coated microspheres, glutaraldehyde or another gelatin hardener. After completion of the crosslinking reaction, the resulting microspheres are recovered from the reaction medium by a conventional means, e.g., decantation, filtration, or centrifugation. The microspheres may be washed and dried in a standard technique, e.g., lyophilization.

The coacervate-coated microspheres of the present invention preferably have a sphere size (mean diameter) of about 0.5  $\mu\text{m}$  to about 100  $\mu\text{m}$ , and contain about 10% to 40% (w/w) of a pharmaceutically active substance. In other words, the weight ratio of the pharmaceutically active substance to the coacervate (walls) in the microspheres on a dry basis is about from 1:9 to about 2:3. The pharmaceutical compositions based on the coacervate-coated microspheres can be utilized for oral ingestion, implantation, or external application to the skin or a mucous membrane. Where implantation is desired, they can be implanted subcutaneously, constitute a part of a prosthesis, or be inserted in a cavity of the human body. Subcutaneous implantation using a syringe consists of

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injecting the implant directly into subcutaneous tissue, and is a particularly effective method of controlled drug delivery. This method is viewed as an alternative to surgery where some risk may be encountered. Thus, the microcapsules obtained in the present invention can be suspended in a physiological buffer and introduced via a syringe to the desired site.

For example, the present compositions incorporating hydrocortisone or any other known anti-inflammatory agent can be injected into the region of an inflammatory joint or muscle. As indicated above, a wide variety of pharmaceutical agents can be included in the present compositions and administered to the body in essentially the same manner. Other than anti-inflammatories, the delivery of narcotic antagonists, contraceptive or anabolic steroids, and anticancer agents can be contemplated. Narcotic antagonists, such as naltrexone, cyclazocine, and naloxone, are therapeutically useful in the postdetoxification stage of rehabilitation of drug-dependent patients. Steroids which can be used are, for example, progesterone and estradiol. Anticancer agents which can be used include cyclophosphamide, 5-fluorouracil (5-FU), doxorubicin, and cisplatin. Additionally, intravaginal or intraureteral implants based on the present compositions may be useful for the delivery of contraceptives and other drugs.

Upon application to the desired part of the body by the desired mode, the pharmaceutical compositions of the present invention provide controlled release of the pharmaceutically active substance by allowing it to diffuse through the coacervate walls of microspheres or by allowing the coacervate to degrade *in vivo* upon contact with body fluids. When the coacervate is degradable in the site (e.g., tissue or joint) where the composition is delivered, the degree of its

degradation (i.e., the release rate of the active substance) can be regulated by the crosslinking degree of the coacervate.

5 The microcapsules of the present invention is premised primarily upon components which are found endogenous to the human body, whether natural or synthetic. The present invention thus makes it possible to employ significantly lesser quantities of non-biological materials than generally used in  
10 preparing controlled release pharmaceutical compositions. This high level of biocompatibility reflects in the absence of cytotoxicity and immunogenicity and the lack of causation of inflammation and foreign body reaction.

15 The following non-limiting examples are provided to further illustrate the present invention.

#### EXAMPLE 1

In this example, the following reagents and solvent were used: Gelatin (type A, 60 bloom; Sigma, St.  
20 Louis, MO), chondroitin-4-sulfate (Sigma), glutaraldehyde (Sigma), collagenase (type VII; Sigma), fluorescein isothiocyanate (FITC; Sigma), sCR1 (T Cell Sciences, Cambridge, MA), 2,2'-azino-di-[3-ethyl benzthioazoline-6-sulfonic acid] (ABTS; Bio-Rad,  
25 Hercules, CA), and sheep red blood cells (Thomas D. Morris, Inc., Philadelphia, PA).

sCR1 (22 mg) was dissolved in 5 ml of 0.2% (w/v) chondroitin sulfate in distilled water at ambient temperature. An equal volume of 5% (w/v) gelatin in  
30 distilled water at 37°C was added to the chondroitin sulfate solution. Upon mixing by a vortex mixer, a suspension was obtained and this was stirred for 10 minutes. Glutaraldehyde (5  $\mu$ l/ml) in distilled water was added to the suspension. The mixture was shaken in  
35 a rotary shaker for 30 minutes at room temperature. Ethanolamine-HCl (pH 9.0, 0.6 ml/ml solution) was added to the mixture in order to quench the excess

glutaraldehyde. The resulting mixture was allowed to stand overnight at 0°C, precipitating microparticles. The microparticles were collected, washed with phosphate buffered saline solution (PBS; 0.01 M, pH 7.4), and dried.

The morphology of the microparticles' surface was determined by scanning electron microscope (SEM). Samples were dried using CO<sub>2</sub> critical point drying and then sputter coated with gold. SEM images were taken at 20 to 30KV on an Amray 1810 microscope. Under SEM, the microparticles were determined to be spherical and to have a particle size of between 5  $\mu$ m and 30  $\mu$ m.

The amount of SCR1 encapsulated in the microspheres was determined by analysis in solution before and after encapsulation. The analysis was conducted according to an ELISA procedure using monoclonal Mouse IgG1 anti-SCR1 and a peroxidase conjugate of Sheep anti-Mouse IgG. ABTS was used as the peroxidase substrate. Thus, a SCR loading level of about 35% (w/w) was determined.

A confocal microscope (Bio-Rad MRC-600 microscope) study was undertaken to determine the distribution of SCR1 within the microsphere.

For this purpose, fluorescein labelled SCR1 was synthesized by addition of 5 mg of FITC (dissolved in 0.5 ml of acetone) to 3.6 mg of SCR1 in 3 nl of 0.1 M sodium bicarbonate. After reaction at room temperature for 15 minutes and at 0°C overnight, the mixture was dialyzed against water and lyophilized.

SCR-FITC was encapsulated substantially in the same manner as that described above. A photomicrograph of the microsphere showed that SCR1-FITC is uniformly distributed throughout the microsphere with a slightly lower concentration toward the outer surface.

A similar analysis using FITC-labelled gelatin showed that the microspheres are of a matrix type and not a core and shell type.

5 sCR1 is a recombinant, soluble, human complement  
receptor 1 (CR1) which has been shown to retain the  
activity of CR1 both *in vitro* and *in vivo* (J.M. Ahearn,  
*et al.*, *Advances in Immunology*, **1989**, 46, 183-219; H.F.  
10 Weisman, *et al.*, *Science*, **1990**, 249, 146-151). CR1 is  
an endogenous regulatory protein that blocks  
proteolytic enzymes which activate complement proteins  
C<sub>3</sub> and C<sub>5</sub>. Administration of sCR1 will interfere with  
the complement activation, thus averting destructive  
15 biological processes which lead to inflammation and  
damage to cartilages. sCR1 is a high molecular weight  
(200 KD) protein with a half life shorter than 15  
minutes at physiological conditions. Thus, the use of  
sCR1 in its native form as an anti-rheumatic agent will  
15 require frequent intra-articular injections.  
Encapsulation of the protein within microspheres can  
provide adequate protection in the joint cavity,  
introduce controlled release features and reduce the  
frequency of intra-articular injections needed.

20

#### EXAMPLE 2

##### IN VITRO DRUG RELEASE FROM MICROSPHERES

Release experiments were conducted in 20 ml of PBS  
containing 0.36 mM calcium chloride and 10 units of  
collagenase. The microspheres prepared according to  
25 the procedure of EXAMPLE 1 (27 mg dry weight) were  
suspended in the PBS solution and incubated at 37°C.  
Control samples were also prepared in the absence of  
collagenase.

Sample aliquots were withdrawn from the incubated  
30 mixture at different times and placed in a tube  
containing 100  $\mu$ l of 0.1 M EDTA solution. Released  
sCR1 was measured by ELISA as described above. The  
activity of sCR1 was determined by the inhibition of  
complement activation as measured by the lysis of  
35 sensitized sheep red blood cells (SRBC) (H.F. Weisman,  
*et al.*, *op. cit.*). The protein was found to maintain  
its ability throughout the release period. The control

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samples showed that no sCR1 was released during one week. The *in vitro* release of sCR1 from the microspheres was thus determined as a function of time. The results of the test are shown in Figures 2 and 3.

5       A model experiment using a biological inactive protein indicated that the protein release was dependent upon the level of collagenase. In a joint cavity where inflammation progresses, the level of collagenase is known to raise (J. Menzel, et al., *Z. Rheumatol.*, 1977, 36, 364-377). Thus, the increased  
10       level of collagenase would facilitate *in vivo* degradation of the microspheres of the present invention in inflammatory lesions.

15       The invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made without departing from the spirit or scope of the invention.

## CLAIMS

1. A pharmaceutical composition for the controlled  
release of a pharmaceutically active substance  
5 comprising a therapeutically effective amount of  
the pharmaceutically active substance, encapsulated  
in the form of microspheres by a coating of a  
crosslinked coacervate of gelatin and chondroitin  
sulfate.
- 10 2. The pharmaceutical composition according to claim  
1, wherein the crosslinked coacervate is  
glutaraldehyde-crosslinked.
3. The pharmaceutical composition according to claim  
1, wherein the microspheres have a sphere size of  
15 from about 0.5  $\mu\text{m}$  to about 100  $\mu\text{m}$ .
4. The pharmaceutical composition according to claim  
1, wherein the pharmaceutically active substance is  
present in an amount of from about 10% to about 40%  
by weight based on the total.
- 20 5. The pharmaceutical composition according to claim  
1, wherein the pharmaceutically active substance is  
selected from the group consisting of analgesics,  
antibacterials, antifungals, immunosuppressants,  
anti-inflammatory agents, anti-cancer agents,  
25 hormones, contraceptives, and narcotic antagonists.
6. The pharmaceutical composition according to claim  
5, wherein the pharmaceutically active substance is  
an anti-inflammatory agent.
7. The pharmaceutical composition according to claim  
30 1, wherein the composition is in the form of an  
intra-articular formulation.
8. The pharmaceutical composition according to claim  
1, wherein the pharmaceutically active substance is  
water-soluble.
- 35 9. The pharmaceutical composition according to claim  
8, wherein the water-soluble substance is a  
proteinaceous drug.



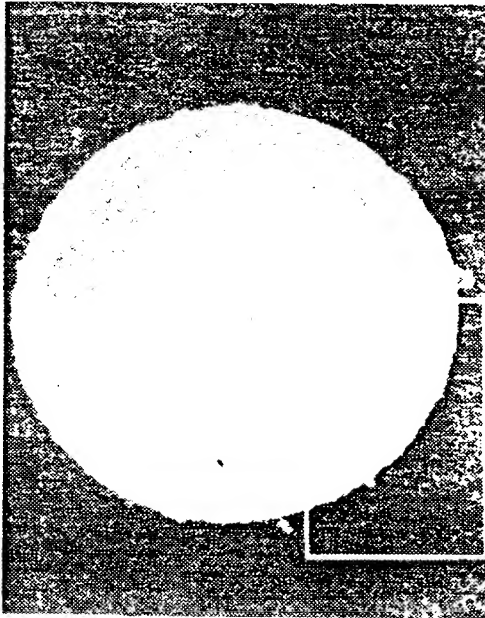
10. A process for preparing the pharmaceutical composition according to claim 1, which comprises the steps of:
- (a) providing a gelatin aqueous solution;
  - 5 (b) providing a chondroitin sulfate aqueous solution;
  - (c) adding a therapeutically effective amount of the pharmaceutically active substance either to the solution in step (a) or to the solution in  
10 step (b);
  - (d) mixing the gelatin and chondroitin sulfate solutions to form a coacervate suspension;
  - (e) adding a crosslinking agent to the coacervate suspension to crosslink the coacervates, the  
15 coacervates encapsulating the pharmaceutically active substance; and
  - (f) incubating the coacervate suspension to form microspheres and recovering the microspheres.
11. The process according to claim 10, wherein gelatin  
20 is present in the aqueous solution in step (a) in a concentration of from about 1% to about 10% (w/v).
12. The process according to claim 10, wherein chondroitin sulfate is present in the aqueous solution in step (b) in a concentration of from  
25 about 0.05% to about 2% (w/v).
13. The process according to claim 10, wherein the gelatin aqueous solution in step (a) contains 5 w/v % gelatin, and the chondroitin sulfate aqueous solution in step (b) contains 0.2 w/v% chondroitin  
30 sulfate.
14. The process according to claim 10, wherein in step (c) the pharmaceutically active substance is added to the chondroitin sulfate solution.
15. The process according to claim 10, wherein in step  
35 (a) equal volumes of the gelatin solution and the chondroitin sulfate solution are mixed.

16. The process according to claim 10, wherein the crosslinking agent is glutaraldehyde.
17. The process according to claim 10, wherein the pharmaceutically active substance is a water-soluble protein.
18. The process according to claim 10, wherein the microspheres have a sphere size of from about 0.5  $\mu\text{m}$  to about 100  $\mu\text{m}$ .
19. A method of releasing a pharmaceutically active substance *in vivo* in a controlled manner which comprises administering the pharmaceutical composition according to claim 1 to a host and degrading the coacervates by the action of a gelatin-digesting enzyme.
20. The method according to claim 19, wherein the crosslinked coacervate is glutaraldehyde-crosslinked.
21. The method according to claim 19, wherein the administration is parenteral.
22. The method according to claim 21, wherein the administration is subcutaneous.
23. The method according to claim 21, wherein the administration is intra-articular.
24. The method according to claim 23, wherein the host is in need of treatment of rheumatoid arthritis.
25. The method according to claim 23, wherein the gelatin-digesting enzyme is collagenase.
26. Encapsulated pharmaceutically active substance in the form of microspheres, the microspheres having a coating of a crosslinked coacervate of gelatin and chondroitin sulfate wherein the weight ratio of the pharmaceutically active substance to the coacervate in the microspheres on a dry basis is from 1:9 to 2:3.

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27. The encapsulated pharmaceutically active substance according to claim 26, wherein the microspheres have a sphere size of from about 0.5  $\mu\text{m}$  to about 100  $\mu\text{m}$ .
- 5 28. The encapsulated pharmaceutically active substance according to claim 26, wherein the crosslinked coacervate is glutaraldehyde-crosslinked.
29. The encapsulated pharmaceutically active substance according to claim 26, wherein the active substance
- 10 is a water-soluble proteinaceous drug.

1/3



Time 0

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1  $\mu\text{m}$ 

FIG.1A



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1  $\mu\text{m}$ 

FIG.1B

2/3

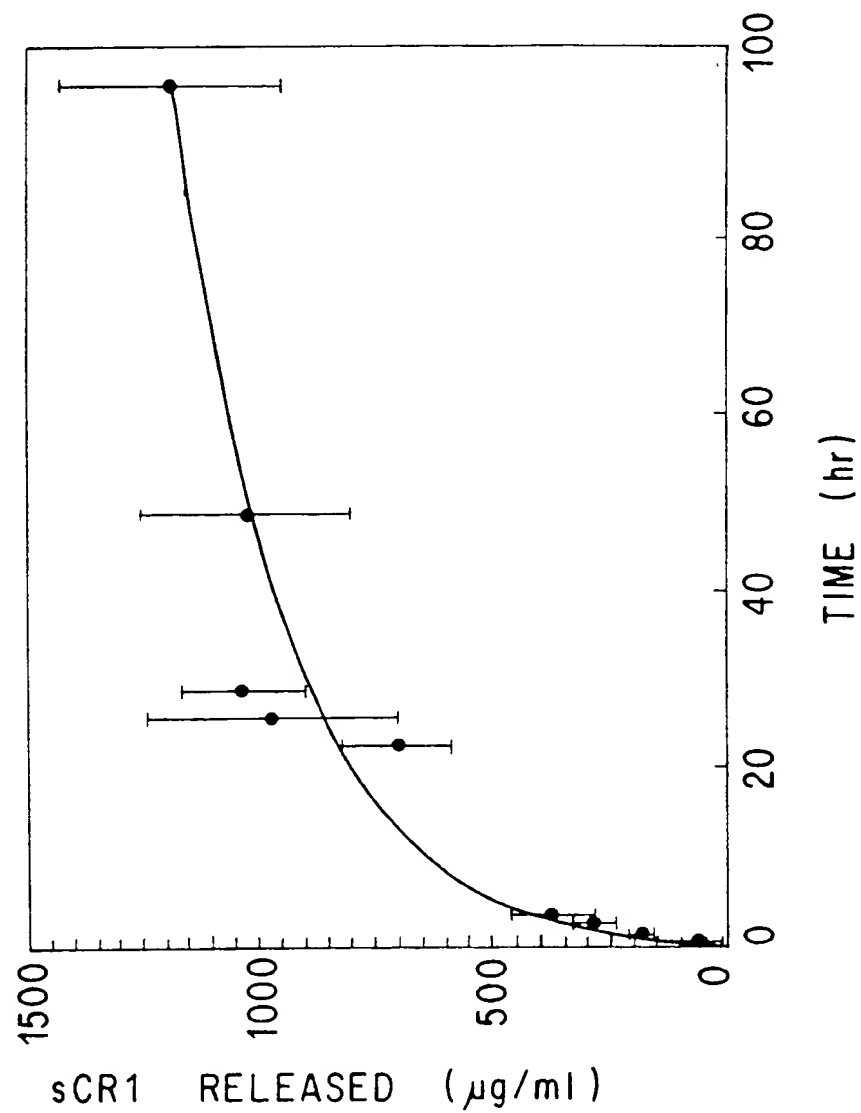


FIG. 2

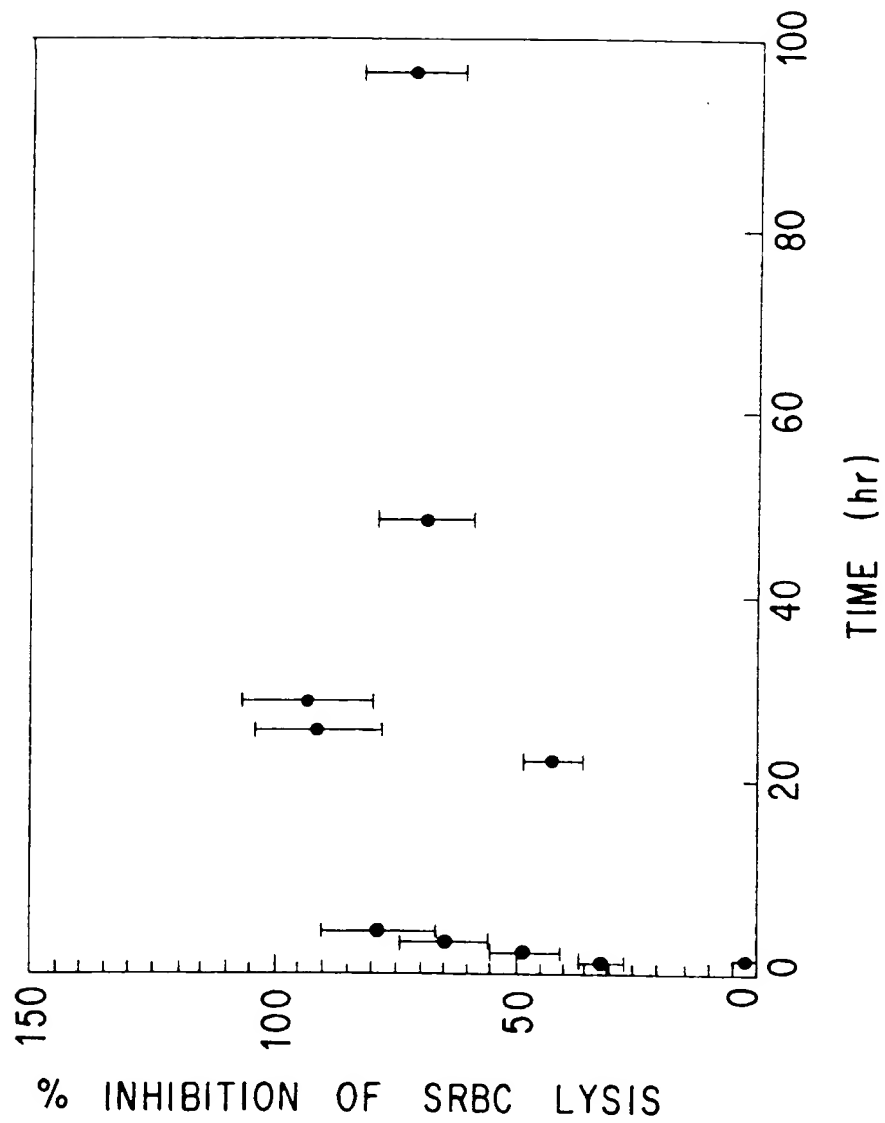


FIG.3

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : A61K 9/16

US CL : 424/490, 492, 493

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/490, 492, 493

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y -- A	US, A, 5,051,304 (DAVID ET AL) 24 September 1991. See the Abstract, see columns 2-4.	1-18,26-29 ----- 19-25
Y, P ----- A, P	US, A, 5,260,002 (WANG) 09 November 1993. See column 2, Table 2 at column 8.	1-18,26-29 ----- 19-25

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

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Date of the actual completion of the international search

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